AMENDMENT

IN THE SPECIFICATION:

On page 1, line 1, please insert the following:

This application is a divisional of U.S. Serial No. 08/537,361, filed October 2, 1995, now U.S. Patent No. 6,121,037, issued September 19, 2000, which is a continuation-in-part of U.S. Serial No. 08/326,670, filed October 18, 1994, now U.S. Patent 5,698,438, issued December 16, 1997. The disclosures of each of these prior applications are considered as being part of the disclosure of the application and are explicitly incorporated by reference herein.

On page 6, line 8, 11 and 12, please enter the following amendments:

In a first aspect, the invention provides a nucleic acid having a nucleotide sequence encoding a bacterial hemoglobin receptor protein gene. In a preferred embodiment, the bacterial hemoglobin receptor protein gene is isolated from bacteria of *Neisseria* species. In a particularly preferred embodiment, the hemoglobin receptor protein gene is isolated from *Neisseria meningitidis*, serotype C. In a particular example of this embodiment, the nucleic acid comprises a 3.3 kilobase (kb) *Bam*HI/*Hin*dIII fragment of *N. meningitidis* genomic DNA. In this embodiment, the nucleotide sequence comprises an open reading frame of 2376 nucleotides of *N. meningitidis* genomic DNA encoding 792 amino acids comprising the hemoglobin receptor gene. In this embodiment of the invention, the nucleotide sequence of the *N. meningitidis* hemoglobin receptor gene is the sequence depicted in ~~Figure 2~~ Figures 2A-2H (SEQ ID No:1). It will be understood that the *N. meningitidis* gene as disclosed herein is defined, insofar as is necessary, by the amino acid sequence of the protein encoded therein, said amino acid sequence being represented in ~~Figure 2~~ Figures 2A-2H (SEQ. ID No.:2). Thus, it will be understood that the particular nucleotide sequence depicted in ~~Figure 2~~ Figures 2A-2H (SEQ. ID. No.:1) is but one of a number of equivalent nucleotide sequences that encode the hemoglobin receptor protein, due to the degeneracy of the genetic code, and that all such alternative, equivalent nucleotide sequences are hereby explicitly encompassed within the disclosed nucleotide sequences of the invention. Also included herein are any mutant or allelic variations of this nucleotide sequence, either naturally occurring or the product of *in vitro* chemical or genetic modification. Each such variant will be understood to have essentially the same nucleotide sequence as the nucleotide sequence of the corresponding *N. meningitidis* hemoglobin receptor protein disclosed herein.

On page 6, lines 21, 22, 23, 25, & 27, and page 7, lines 1 and 2, please enter the following amendments:

In another particularly preferred embodiment of this aspect of the invention, the hemoglobin receptor protein gene is isolated from *Neisseria meningitidis*, ~~serotype A~~ serotype B. In a particular example of this embodiment, the nucleic acid comprises a ~~2373~~ 2376 basepair (bp) polymerase chain reaction-amplified fragment of *N. meningitidis*, ~~serotype A~~ serotype B genomic DNA. In this embodiment, the nucleotide sequence comprises an open reading frame of 2373 nucleotides of *N. meningitidis* genomic DNA encoding ~~790~~ 791 amino acids comprising the hemoglobin receptor gene. In this embodiment of the invention, the nucleotide sequence of the *N. meningitidis* hemoglobin receptor gene is the sequence depicted in ~~Figure 7~~ Figures 7A-7I (SEQ ID No:3). It will be understood that the *N. meningitidis* gene as disclosed herein is defined, insofar as is necessary, by the amino acid sequence of the protein encoded therein, said amino acid sequence being represented in ~~Figure 7~~ Figures 7A-7I (SEQ. ID No.:4). Thus, it will be understood that the particular nucleotide sequence depicted in ~~Figure 7~~ Figures 7A-7I (SEQ. ID. No.:3) is but one of a number of equivalent nucleotide sequences that encode the hemoglobin receptor protein, due to the degeneracy of the genetic code, and that all such alternative, equivalent nucleotide sequences are hereby explicitly encompassed within the disclosed nucleotide sequences of the invention. Also included herein are any mutant or allelic variations of this nucleotide sequence, either naturally occurring or the product of *in vitro* chemical or genetic modification. Each such variant will be understood to have essentially the same nucleotide sequence as the nucleotide sequence of the corresponding *N. meningitidis* hemoglobin receptor protein disclosed herein.

On page 7, lines 11, 12, 13, 14, 15, 17, 20 and 21, please enter the following amendments:

In another particularly preferred embodiment of this aspect of the invention, the hemoglobin receptor protein gene is isolated from *Neisseria meningitidis*, ~~serotype B~~ serotype A. In a particular example of this embodiment, the nucleic acid comprises a ~~2376~~ 2373 basepair (bp) polymerase chain reaction-amplified fragment of *N. meningitidis*, ~~serotype B~~ serotype A genomic DNA. In this embodiment, the nucleotide sequence comprises an open reading frame of ~~2373~~ 2370 nucleotides of *N. meningitidis* genomic DNA encoding ~~791~~ 790 amino acids comprising the hemoglobin receptor gene. In this embodiment of the invention, the nucleotide sequence of the *N. meningitidis* hemoglobin receptor gene is the sequence depicted in ~~Figure 8~~ Figures 8A-8I (SEQ ID No:5). It will be understood that the *N. meningitidis* gene as disclosed herein is defined, insofar as is necessary, by the amino acid sequence of the protein encoded therein, said amino acid sequence being represented in ~~Figure 8~~ Figures 8A-8I (SEQ. ID No.:6). Thus, it will be understood that the particular

nucleotide sequence depicted in ~~Figure 8~~ Figures 8A-8I (SEQ. ID. No.:5) is but one of a number of equivalent nucleotide sequences that encode the hemoglobin receptor protein, due to the degeneracy of the genetic code, and that all such alternative, equivalent nucleotide sequences are hereby explicitly encompassed within the disclosed nucleotide sequences of the invention. Also included herein are any mutant or allelic variations of this nucleotide sequence, either naturally occurring or the product of *in vitro* chemical or genetic modification. Each such variant will be understood to have essentially the same nucleotide sequence as the nucleotide sequence of the corresponding *N. meningitidis* hemoglobin receptor protein disclosed herein.

On page 8, lines 5, 6, 8, 10 and 12, please enter the following amendments:

In yet other preferred embodiments, the invention provides nucleic acid encoding a hemoglobin receptor protein gene isolated from *Neisseria gonorrhoeae*. In a particular example of this embodiment, the nucleic acid comprises a 2378 basepair (bp) polymerase chain reaction-amplified fragment of *N. gonorrhoeae* genomic DNA. In this embodiment, the nucleotide sequence comprises an open reading frame of ~~2373~~ 2370 nucleotides of *N. gonorrhoeae* genomic DNA encoding ~~791~~ 790 amino acids comprising the hemoglobin receptor gene. In this embodiment of the invention, the nucleotide sequence of the *N. gonorrhoeae* hemoglobin receptor gene is the sequence depicted in ~~Figure 9~~ Figures 9A-9I (SEQ ID No:7). It will be understood that the *N. gonorrhoeae* gene as disclosed herein is defined, insofar as is necessary, by the amino acid sequence of the protein encoded therein, said amino acid sequence being represented in ~~Figure 9~~ Figures 9A-9I (SEQ. ID No.:8). Thus, it will be understood that the particular nucleotide sequence depicted in ~~Figure 9~~ Figures 9A-9I (SEQ. ID. No.:7) is but one of a number of equivalent nucleotide sequences that encode the hemoglobin receptor protein, due to the degeneracy of the genetic code, and that all such alternative, equivalent nucleotide sequences are hereby explicitly encompassed within the disclosed nucleotide sequences of the invention. Also included herein are any mutant or allelic variations of this nucleotide sequence, either naturally occurring or the product of *in vitro* chemical or genetic modification. Each such variant will be understood to have essentially the same nucleotide sequence as the nucleotide sequence of the corresponding *N. gonorrhoeae* hemoglobin receptor protein disclosed herein.

On page 8, lines 26-29, and page 9, lines 1-5, 7 & 8, please enter the following amendments:

The invention also provides bacterial hemoglobin receptor proteins. In a preferred embodiment, the bacterial hemoglobin receptor protein is isolated from bacteria of *Neisseria* species. In a particularly preferred embodiment, the hemoglobin receptor protein is isolated from *Neisseria*

meningitidis. In a particular example of this embodiment, the protein is derived from *N. meningitidis*, serotype C and comprises an amino acid sequence of 792 amino acids. In this embodiment of the invention, the amino acid sequence of the *N. meningitidis*, serotype C hemoglobin receptor protein is the sequence depicted in ~~Figure 2~~ Figures 2A-2H (SEQ ID No:2). In another example of this embodiment, the protein is derived from *N. meningitidis*, ~~serotype A~~ serotype B and comprises an amino acid sequence of ~~790~~ 791 amino acids. In this embodiment of the invention, the amino acid sequence of the *N. meningitidis*, ~~serotype A~~ serotype B hemoglobin receptor protein is the sequence depicted in ~~Figure 7~~ Figures 7A-7I (SEQ ID No:4). In yet another example of this embodiment, the protein is derived from *N. meningitidis*, ~~serotype B~~ serotype A and comprises an amino acid sequence of ~~791~~ 790 amino acids. In this embodiment of the invention, the amino acid sequence of the *N. meningitidis*, ~~serotype B~~ serotype A hemoglobin receptor protein is the sequence depicted in ~~Figure 8~~ Figures 8A-8I (SEQ ID No:6). The invention also provides hemoglobin receptor protein derived from *N. gonorrhoeae*. In this embodiment of the invention, the protein comprises an amino acid sequence of ~~791~~ 790 amino acids, and the amino acid sequence of the *N. gonorrhoeae* hemoglobin receptor protein is the sequence depicted in ~~Figure 9~~ Figures 9A-9I (SEQ ID No:8). Also explicitly encompassed within the scope of this invention are related bacterial hemoglobin receptor proteins, particularly such proteins isolated from *Neisseria* species, having essentially the same amino acid sequence and substantially the same biological properties as the hemoglobin receptor protein encoded by the *N. meningitidis* and *N. gonorrhoeae* nucleotide sequences described herein.

On page 9, lines 24, 25, 27-29 & 32, and page 10, lines 1 and 5, please enter the following amendments:

In another aspect, the invention provides a homogeneous preparation of an approximately 85.5 kiloDalton (kD) bacterial hemoglobin receptor protein or derivative thereof, said size being understood to be the size of the protein before any post-translational modifications thereof. Also provided is a 90 kD embodiment of the receptor as determined by sodium dodecyl sulfate/polyacrylamide gel electrophoresis under reducing conditions. In a preferred embodiment, the bacterial hemoglobin receptor protein is isolated from bacteria of *Neisseria* species. In a particularly preferred embodiment, the hemoglobin receptor protein is isolated from *Neisseria meningitidis*. In one embodiment of this aspect of the invention, the protein is isolated from *N. meningitidis*, serotype C and the amino acid sequence of the bacterial hemoglobin receptor protein or derivative thereof preferably is the amino acid sequence of the hemoglobin receptor protein shown in ~~Figure 2~~ Figures 2A-2H (SEQ ID No:2). In a second embodiment of this aspect of the invention, the protein is isolated from *N. meningitidis*, ~~serotype A~~ serotype B and the amino acid sequence of the bacterial hemoglobin receptor protein or derivative thereof preferably is the amino acid sequence

of the hemoglobin receptor protein shown in ~~Figure 7~~ Figures 7A-7I (SEQ ID No:4). In a third embodiment of this aspect of the invention, the protein is isolated from *N. meningitidis*, ~~serotype B~~ serotype A and the amino acid sequence of the bacterial hemoglobin receptor protein or derivative thereof preferably is the amino acid sequence of the hemoglobin receptor protein shown in ~~Figure 8~~ Figures 8A-8I (SEQ ID No:6). The invention also provides a homogeneous preparation of a bacterial hemoglobin receptor protein isolated from *N. gonorrhoeae*. In a preferred embodiment, the amino acid sequence of the bacterial hemoglobin receptor protein or derivative thereof preferably is the amino acid sequence of the hemoglobin receptor protein shown in ~~Figure 9~~ Figures 9A-9I (SEQ ID No:8).

On page 12, lines 5, 8, 11, 12, 14, 16 and 20, please enter the following amendments:

The present invention provides recombinant expression constructs comprising a nucleic acid encoding a bacterial hemoglobin receptor protein wherein the construct is capable of expressing the encoded hemoglobin receptor protein in cultures of cells transformed with the construct. Preferred embodiments of such constructs comprise the *N. meningitidis*, serotype C hemoglobin receptor gene depicted in ~~Figure 2~~ Figures 2A-2H (SEQ ID No.:1), such constructs being capable of expressing the bacterial hemoglobin receptor protein encoded therein in cells transformed with the construct. Additional preferred embodiments of such constructs comprise the *N. meningitidis*, ~~serotype A~~ serotype B hemoglobin receptor gene depicted in ~~Figure 7~~ Figures 7A-7I (SEQ ID No.:3), such constructs being capable of expressing the bacterial hemoglobin receptor protein encoded therein in cells transformed with the construct. Further additional preferred embodiments of such constructs comprise the *N. meningitidis*, ~~serotype B~~ serotype A hemoglobin receptor gene depicted in ~~Figure 8~~ Figures 8A-8I (SEQ ID No.:5), such constructs being capable of expressing the bacterial hemoglobin receptor protein encoded therein in cells transformed with the construct. The invention also provides recombinant expression constructs encoding a hemoglobin receptor protein gene isolated from ~~ZN.~~ *N. gonorrhoeae*. In a particularly preferred embodiment, such constructs comprise the *N. gonorrhoeae* hemoglobin receptor gene depicted in ~~Figure 9~~ Figures 9A-9I (SEQ ID No.:7), the constructs being capable of expressing the bacterial hemoglobin receptor protein encoded therein in cells transformed with the construct.

On page 13, lines 18, 20 & 26, and page 14, lines 1, 2, 4, 9, 10 & 12, please enter the following amendments:

In yet another embodiment of this aspect of the invention are provided diagnostic reagents and methods for using such reagents wherein said reagents are nucleic acid hybridization probes

comprising a bacterial hemoglobin receptor gene. In a preferred embodiment, the bacterial hemoglobin receptor protein gene is isolated from bacteria of *Neisseria* species. In a particularly preferred embodiment, the hemoglobin receptor protein gene is isolated from *Neisseria meningitidis*. In particular examples of this embodiment of the invention, the nucleic acid probes comprise a specifically-hybridizing fragment of a 3.3 kilobase (kb) *Bam*HI/*Hin*dIII fragment of *N. meningitidis*, serotype C genomic DNA. In this embodiment, the nucleotide sequence comprises all or a specifically-hybridizing fragment of an open reading frame of 2376 nucleotides of *N. meningitidis*, serotype C genomic DNA encoding 792 amino acids comprising the hemoglobin receptor gene. In this embodiment of the invention, the nucleotide sequence of the *N. meningitidis*, serotype C hemoglobin receptor gene is the sequence depicted in ~~Figure 2~~ Figures 2A-2H (SEQ ID No:1). In another example of this embodiment of the invention, the nucleic acid probes comprise a specifically-hybridizing fragment of a ~~2373~~ 2376 bp, polymerase chain reaction-amplified fragment of *N. meningitidis*, ~~serotype A~~ serotype B genomic DNA. In this embodiment, the nucleotide sequence comprises all or a specifically-hybridizing fragment of an open reading frame of 2373 nucleotides of *N. meningitidis*, ~~serotype A~~ serotype B genomic DNA encoding ~~790~~ 791 amino acids comprising the hemoglobin receptor gene. In this embodiment of the invention, the nucleotide sequence of the *N. meningitidis*, serotype A hemoglobin receptor gene is the sequence depicted in ~~Figure 7~~ Figures 7A-7I (SEQ ID No:3). In yet another example of this embodiment of the invention, the nucleic acid probes comprise a specifically-hybridizing fragment of a ~~2376~~ 2373 bp, polymerase chain reaction-amplified fragment of *N. meningitidis*, ~~serotype B~~ serotype A genomic DNA. In this embodiment, the nucleotide sequence comprises all or a specifically-hybridizing fragment of an open reading frame of ~~2373~~ 2370 nucleotides of *N. meningitidis*, serotype A genomic DNA encoding ~~791~~ 790 amino acids comprising the hemoglobin receptor gene. In this embodiment of the invention, the nucleotide sequence of the *N. meningitidis*, ~~serotype B~~ serotype A hemoglobin receptor gene is the sequence depicted in ~~Figure 8~~ Figures 8A-8I (SEQ ID No:5). The invention also provides nucleic acid hybridization probes comprising a bacterial hemoglobin receptor gene isolated from *N. gonorrhoeae*. In a preferred embodiment of this aspect of the invention, the nucleic acid probes comprise a specifically-hybridizing fragment of a ~~2373~~ 2370 bp, polymerase chain reaction-amplified fragment of *N. gonorrhoeae* genomic DNA. In this embodiment, the nucleotide sequence comprises all or a specifically-hybridizing fragment of an open reading frame of 2370 nucleotides of *N. gonorrhoeae* genomic DNA encoding ~~791~~ 790 amino acids comprising the hemoglobin receptor gene. In this embodiment of the invention, the nucleotide sequence of the *N. gonorrhoeae* hemoglobin receptor gene is the sequence depicted in ~~Figure 9~~ Figures 9A-9I (SEQ ID No:7). It will be understood that the term "specifically-hybridizing" when used to describe a fragment of a nucleic acid encoding a bacterial hemoglobin receptor gene is intended to mean that nucleic acid hybridization of such a fragment is stable under high stringency conditions of hybridization and

washing as the term "high stringency" would be understood by those having skill in the molecular biological arts.

On page 15, lines 15, 17, 18, 20, 22, 23, 24, 25 & 27-29, and page 16, lines 1, 8 & 9, please enter the following amendments:

In yet another embodiment of this aspect of the invention are provided therapeutic reagents and methods for using such reagents wherein said reagents comprise recombinant expression constructs of the invention, or a homologue thereof that expresses the nucleic acid encoding a hemoglobin receptor in an antisense orientation. In a preferred embodiment, the bacterial hemoglobin receptor protein gene is isolated from bacteria of *Neisseria* species. In a particularly preferred embodiment, the hemoglobin receptor protein gene is isolated from *Neisseria meningitidis*. In particular examples of this embodiment of the invention, the nucleic acids comprise a specifically-hybridizing fragment of a 3.3 kilobase (kb) *Bam*HI/*Hin*dIII fragment of *N. meningitidis*, serotype C genomic DNA. In this embodiment, the nucleotide sequence comprises all or a specifically-hybridizing fragment of an open reading frame of 2376 nucleotides of *N. meningitidis*, serotype C genomic DNA encoding 792 amino acids comprising the hemoglobin receptor gene. In this embodiment of the invention, the nucleotide sequence of the *N. meningitidis*, serotype C hemoglobin receptor gene is the sequence depicted in ~~Figure 2~~ Figures 2A-2H (SEQ ID No:1). In another example of this embodiment of the invention, the nucleic acid probes comprise a specifically-hybridizing fragment of a ~~2373~~ 2376 bp, polymerase chain reaction-amplified fragment of *N. meningitidis*, ~~serotype A~~ serotype B genomic DNA. In this embodiment, the nucleotide sequence comprises all or a specifically-hybridizing fragment of an open reading frame of ~~2370~~ 2373 nucleotides of *N. meningitidis*, ~~serotype A~~ serotype B genomic DNA encoding ~~790~~ 791 amino acids comprising the hemoglobin receptor gene. In this embodiment of the invention, the nucleotide sequence of the *N. meningitidis*, ~~serotype A~~ serotype B hemoglobin receptor gene is the sequence depicted in ~~Figure 7~~ Figures 7A through 7I (SEQ ID No:3). In yet another example of this embodiment of the invention, the nucleic acid probes comprise a specifically-hybridizing fragment of a ~~2376~~ 2373 bp, polymerase chain reaction-amplified fragment of *N. meningitidis*, ~~serotype B~~ serotype A genomic DNA. In this embodiment, the nucleotide sequence comprises all or a specifically-hybridizing fragment of an open reading frame of ~~2373~~ 2370 nucleotides of *N. meningitidis*, ~~serotype B~~ serotype A genomic DNA encoding ~~791~~ 790 amino acids comprising the hemoglobin receptor gene. In this embodiment of the invention, the nucleotide sequence of the *N. meningitidis*, ~~serotype B~~ serotype A hemoglobin receptor gene is the sequence depicted in ~~Figure 8~~ Figures 8A through 8I (SEQ ID No:5). The invention also provides recombinant expression constructs of the invention, or a homologue thereof that expresses the nucleic acid encoding a

hemoglobin receptor in an antisense orientation, wherein the nucleic acid encodes a bacterial hemoglobin receptor gene isolated from *N. gonorrhoeae*. In a preferred embodiment of this aspect of the invention, the nucleic acid probes comprise a specifically-hybridizing fragment of a ~~2373~~ <u>2370</u> bp, polymerase chain reaction-amplified fragment of *N. gonorrhoeae* genomic DNA. In this embodiment, the nucleotide sequence comprises all or a specifically-hybridizing fragment of an open reading frame of ~~2373~~ <u>2370</u> nucleotides of *N. gonorrhoeae* genomic DNA encoding ~~791~~ <u>790</u> amino acids comprising the hemoglobin receptor gene. In this embodiment of the invention, the nucleotide sequence of the *N. gonorrhoeae* hemoglobin receptor gene is the sequence depicted in ~~Figure 9~~ <u>Figures 9A - 9I</u> (SEQ ID No:7).

On page 16, line 22, please enter the following amendments:

The present invention is also useful for the detection of bactericidal and/or bacteriostatic analogues, agonists or antagonists, ~~known or unknown~~ <u>recognized or unrecognized</u> of a bacterial hemoglobin receptor protein, preferably derived from bacteria of *Neisseria* species, most preferably isolated from *N. meningitidis*, wherein such compounds are either naturally occurring or embodied as a drug.

On page 17, lines 17 & 24, page 18, lines 9-16, 19, 20 & 26, please enter the following amendments:

~~Figure 2~~ <u>Figures 2A-2H</u> illustrates the nucleotide (SEQ ID No.:1) and deduced amino acid (SEQ ID No.:2) sequences of the *N. meningitidis* hemoglobin receptor protein encoded in a 3.3 kb *Bam*HI/*Hin*dIII DNA fragment.

Figure 3 presents a photograph of a stained SDS/ 10% PAGE electrophoresis gel showing the results of in vitro expression of the *N. meningitides* hemoglobin receptor gene product as an approximately 90 kilodalton protein, and .beta.-lactamase protein having a molecular weight of about 30.0 kilodaltons used as a molecular weight marker.

~~Figure 4~~ <u>Figures 4A-4C</u> presents an amino acid sequence comparison between portions of the N. meningitidis transferrin receptor Tbp1 (SEQ ID No.:9), the *N. meningitdis* lactoferrin receptor LbpA (SEQ ID No.:10), and *N. meningitidis* hemoglobin receptor HmbR (SEQ ID No.:2).

Figure 5 illustrates Southern hybridization analysis of chromosomal DNA from N. meningitidis 8013 and the MC8013hmbR mutant using a *Bam*HI-*Sal*I fragment of the hmb gene as probe labeled using a DIG nonradioactive DNA labelling and detection kit (Boehringer Mannheim Biochemicals, Indianapolis, Ind.). Lane 1 contains DNA from *N. meningitidis* strain MC8013, digested with *ClaI*; lane 2 is MC803hmbR DNA digested with *Cla*I; lane 3, is MC8013 DNA digested with *Bam*HI and *Sal*I; and lane 4 is MC8013hmbR DNA digested with *Bam*HI and *Sal*I.

Figure 6 is a graph describing the course of infection using *N. meningitidis* wild type (MC8013) and hmbR mutant strains in an in vivo rat infant infection model. Each strain was injected intraperitoneally (2 x 10^6 CFU) into three infant inbred Lewis rats. The results represent the average of two similarly-performed experiments.

~~Figure 7~~ <u>Figures 7A-7I</u> illustrates the nucleotide (SEQ ID No.:3) and deduced amino acid (SEQ ID No.:4) sequences of the *N. meningitidis*, ~~serotype A~~ <u>serotype B</u> hemoglobin receptor protein encoded on a ~~2373~~ <u>2376</u> bp polymerase chain reaction-amplified DNA fragment.

~~Figure 8~~ <u>Figures 8A-8I</u> illustrates the nucleotide (SEQ ID No.:5) and deduced amino acid (SEQ ID No.:6) sequences of the *N. meningitidis*, ~~serotype B~~ <u>serotype A</u> hemoglobin receptor protein encoded on a ~~2376~~ <u>2373</u> bp polymerase chain reaction-amplified DNA fragment.

~~Figure 9~~ <u>Figures 9A-9I</u> illustrates the nucleotide (SEQ ID No.:7) and deduced amino acid (SEQ ID No.:8) sequences of the *N. gonorrhoeae* hemoglobin receptor protein encoded on a ~~2376~~ <u>2378</u> bp polymerase chain reaction-amplified DNA fragment.

Figure 10 represents a schematic of a nucleic acid sequence comparison between the hemoglobin receptor proteins derived from *N. meningitidis*, ~~serotype A~~ <u>serotype B</u> (SEQ ID No.:3), ~~serotype B~~ <u>serotype A</u> (SEQ ID No.:5) and <u>serotype</u> C (SEQ ID No.:1) and from *N. gonorrhoeae* (SEQ ID No.:7), wherein the direction of transcription of the genes is in the direction of the arrow, and the following abbreviations refer to restriction endonuclease sites: H represents *Hin*dIII; N represents *Not*I; Bg represents *Bgl*I; Bs represents *Bss*HI; Nr represents *Nru*I; Cl represents *Cla*I; P represents *PstI*; Sa represents *Sac*I; Av represents *AvaI*; B represents *Bam*HI; S represents *Sal*I; EV represents *Eco*RV; Sh represents *Sph*I; and Sy represents *Sty*I.

~~Figure 11~~ Figures 11A-11D presents an amino acid sequence comparison between the hemoglobin receptor proteins derived from *N. meningitidis*, ~~serotypes A~~ serotype B (SEQ ID No.:4), ~~serotype B~~ serotype A (SEQ ID No.:6) and serotype C (SEQ ID No.:2) and from *N. gonorrhoeae* (SEQ ID No.:8).

On page 19, lines 7, 8 & 10, please enter the following amendments:

The term "bacterial hemoglobin receptor" as used herein refers to bacterial proteins comprising the outer membrane of Gram negative bacteria, which specifically mediate transit of hemoglobin-derived hemin, as well as hemin from other sources, through the outer membrane of such bacteria and into the periplasmic space. The bacterial hemoglobin receptor proteins of the invention are characterized by, first, an amino acid sequence that is essentially the sequence depicted in ~~Figure 2~~ Figures 2A-2H (SEQ ID No.:2), ~~Figure 7~~ Figures 7A-7I (SEQ ID No.:4), ~~Figure 8~~ Figures 8A-8I (SEQ ID No.:6) and ~~Figure 9~~ Figures 9A-9I (SEQ ID No.:8). The bacterial hemoglobin receptor proteins of the invention are further characterized by having substantially the same biological activity as a protein having the amino acid sequence depicted in ~~Figure 2~~ Figures 2A-2H (SEQ ID No.:2), ~~Figure 7~~ Figures 7A-7I (SEQ ID No.:4), ~~Figure 8~~ Figures 8A-8I (SEQ ID No.:6) and ~~Figure 9~~ Figures 9A-9I (SEQ ID No.:8). This definition is intended to encompass naturally-occurring variants and mutant proteins, as well as genetically engineered variants made by man.

On page 19, line 19 & 20, please enter the following amendments:

The nucleic acid hybridization probes provided by the invention comprise DNA or RNA having all or a specifically-hybridizing fragment of the nucleotide sequence of the hemoglobin receptor protein as depicted in ~~Figure 2~~ Figures 2A-2H (SEQ ID No.:1), ~~Figure 7~~ Figures 7A-7I (SEQ ID No.:3), ~~Figure 8~~ Figures 8A-8I (SEQ ID No.:5) and ~~Figure 9~~ Figures 9A-9I (SEQ ID No.:7), or any portion thereof effective in nucleic acid hybridization. Mixtures of such nucleic acid hybridization probes are also within the scope of this embodiment of the invention. Nucleic acid probes as provided herein are useful for detecting the presence of a bacteria, *inter alia*, in a human as the result of an infection, in contaminated biological samples and specimens, in foodstuffs and water supplies, or in any substance that may come in to contact with the human. Specific hybridization will be understood to mean that the nucleic acid probes of the invention are capable of forming stable, specific hybridization to bacterially-derived DNA or RNA under conditions of high stringency, as the term "high stringency" would be understood by those with skill in the art (see, for example, Sambrook *et al.*, 1989, Molecular Cloning: A Laboratory Manual, Cold Spring Harbor

Laboratory Press, Cold Spring Harbor, N.Y. and Hames and Higgins, eds., 1985, Nucleic Acid Hybridization, IRL Press, Oxford, U.K.). Hybridization will be understood to be accomplished using well-established techniques, including but not limited to Southern blot hybridization, Northern blot hybridization, in situ hybridization and Southern hybridization to polymerase chain reaction product DNAs. The invention will thus be understood to provide oligonucleotides, specifically, pairs of oligonucleotides, for use as primers in support of *in vitro* amplification of bacterial hemoglobin receptor genes and mRNA transcripts.

On page 20, line 15, please enter the following amendments:

DNA encoding a bacterial hemoglobin receptor protein <u>can be prepared,</u> in view of the instant disclosure, by chemical synthesis, by screening reverse transcripts of mRNA from appropriate cells, by screening genomic libraries from appropriate cells, or by combinations of these procedures, as illustrated below. Screening of mRNA or genomic DNA may be carried out with oligonucleotide probes generated from the nucleic acid sequence information from the bacterial hemoglobin receptor protein disclosed herein. Probes may be labeled with a detectable group such as a fluorescent group, a radioactive atom or a chemiluminescent group in accordance with know procedures and used in conventional hybridization assays, as described in greater detail in the Examples below. In the alternative, bacterial hemoglobin receptor protein-encoding nucleic acids may be obtained by use of the polymerase chain reaction (PCR) procedure, using appropriate pairs of PCR oligonucleotide primers corresponding to nucleic acid sequence information derived from a bacterial hemoglobin receptor protein as provided herein. See U.S. Pat. No. 4,683,195 to Mullis *et al.* and U.S. Pat. No. 4,683,202 to Mullis, as specifically disclosed herein in Example 9 below. In another alternative, such bacterial hemoglobin receptor protein-encoding nucleic acids may be isolated from auxotrophic cells transformed with a bacterial hemoglobin receptor protein gene, thereby relieved of the nutritional requirement for uncomplexed iron (III).

On page 23, line 12, please enter the following amendments:

Preparation of vaccines which contain polypeptide or polynucleotide sequences as active ingredients is well understood in the art. Typically, such vaccines are prepared as injectables, either as liquid solutions or suspensions. However, solid forms suitable for solution in, or suspension in, liquid prior to injection may also be prepared. The preparation may also be emulsified. The active immunogenic ingredient is often mixed with excipients which are pharmaceutically acceptable and compatible with the active ingredient. Suitable excipients are, for example, water, saline, dextrose, glycerol, ethanol, or the like and combinations thereof. In addition, if desired, the vaccine may

contain minor amounts of auxiliary substances such as wetting or emulsifying agents, pH buffering agents, or adjuvants which enhance the effectiveness of the vaccine. The vaccines are conventionally administered parenterally, by injection, for example, either subcutaneously or intramuscularly. Additional formulations which are suitable for other modes of administration include suppositories and, in some cases, oral formulations. For suppositories, traditional binders and carriers may include, for example, polyalkalene glycols or triglycerides; such suppositories may be formed from mixtures containing the active ingredient in the range of 0.5% to 10%, preferably 1 to 2%. Oral formulations include such normally employed excipients as, for example, pharmaceutical grades of ~~manitol~~ mannitol, lactose, starch, magnesium stearate, sodium saccharine, cellulose, magnesium carbonate and the like. These compositions take the form of solutions, suspensions, tablets, pills, capsules, sustained release formulations or powders and contain 10% to 95 % of active ingredient, preferably 25 to70%.

On page 23, line 17, please enter the following amendments:

The polypeptides of the invention may be formulated into the vaccine as neutral or salt forms. Pharmaceutically acceptable ~~salts,~~ salts include the acid additional salts (formed with the free amino groups of the peptide) and which are formed with inorganic acids such as, for example, hydrochloric or phosphoric acids, or such organic acids as acetic, oxalic, tartaric, mandelic, and the like. Salts formed with the free carboxyl groups may also be derived from inorganic bases such as, for example, sodium, potassium, ammonium, calcium, or ferric hydroxides, and such organic bases as isopropylamine, trimethylamine, 2-ethylamino ethanol, histidine, procaine, and the like.

On page 24, lines 1 & 12, please enter the following amendments:

In another embodiment, such vaccines are provided wherein the bacterial hemoglobin receptor proteins or peptide fragments thereof are present in the intact cell membranes of cells expressing such proteins in accordance with the present invention. In preferred embodiments, cells useful in these embodiments include attenuated varieties of cells adapted to growth in humans. Most preferably, said cells are attenuated varieties of cells adapted for growth in humans, *i.e.*, wherein such cells do not cause frank disease or other pathological conditions, such as ~~bactermia~~ bacteremia, endotoxemia or sepsis. For the purposes of this invention, "attenuated" cells will be understood to encompass prokaryotic and eukaryotic cells that do not cause infection, disease, septicemia, endotoxic shock, pyrogenic shock, or other serious and adverse reactions to administration of vaccines to an animal, most preferably a human, when such cells are introduced into the animal, whether such cells are viable, living, heat-, chemically- or genetically attenuated or inactivated, or

dead. It will be appreciated by those with skill in this art that certain minor side-effects of vaccination, such as short-term fever, muscle discomfort, general malaise, and other well-known reactions to vaccination using a variety of different types of vaccines, can be anticipated as accompanying vaccination of an animal, preferably a human, using the vaccines of the invention. Such acute, short-term and non-life-threatening side effects are encompassed in the instant definition of the vaccines of the invention, and vaccines causing such side-effects fall within the definition of "attenuated" presented herein. Preferred examples of such attenuated cells include ~~attenutated~~ attenuated varieties of Salmonella species, preferably Salmonella typhi and Salmonella typhimurium, as well as other attenuated bacterial species. It will be specifically understood that these embodiments of the vaccines of the invention encompass so-called "live" attenuated cell preparations as well as heat- or chemically-inactivated cell preparations.

On page 27, line 16, please enter the following amendments:

Hybridoma cell lines are made by fusing individual cells of a myeloma cell line with spleen cells derived from animals immunized with a homogeneous preparation of a bacterial hemoglobin receptor protein, membranes comprised thereof, cells expressing such protein, or epitopes of a bacterial hemoglobin receptor protein, used per se or comprising a heterologous or fusion protein construct, as described above. ~~The~~ Preferred myeloma cell lines used in the invention include lines derived from myelomas of mice, rats, hamsters, primates and humans. Preferred myeloma cell lines are from mouse, and the most preferred mouse myeloma cell line is P3X63-Ag8.653. The animals from whom spleens are obtained after immunization are rats, mice and hamsters, preferably mice, most preferably Balb/c mice. Spleen cells and myeloma cells are fused using a number of methods well known in the art, including but not limited to incubation with inactivated Sendai virus and incubation in the presence of polyethylene glycol (PEG). The most preferred method for cell fusion is incubation in the presence of a solution of 45% (w/v) PEG-1450. Monoclonal antibodies produced by hybridoma cell lines can be harvested from cell culture supernatant fluids from in vitro cell growth; alternatively, hybridoma cells can be injected subcutaneously and/or into the peritoneal cavity of an animal, most preferably a mouse, and the monoclonal antibodies obtained from blood and/or ascites fluid.

On page 28, line 26, please enter the following amendments:

Also provided by the present invention are diagnostic and therapeutic methods of detecting and treating an infection in a human, by ~~a pathogenic~~ pathogenic organisms expressing a bacterial hemoglobin receptor protein. Diagnostic reagents for use in such methods include the antibodies, most preferably monoclonal antibodies, of the invention. Such antibodies are used in conventional

immunological techniques, including but not limited to enzyme-linked immunosorbent assay (ELISA), radioimmune assay (RIA), Western blot assay, immunological titration assays, immunological diffusion assays (such as the Ouchterlony assay), and others known to those of skill in the art. Also provided are epitopes derived from a bacterial hemoglobin receptor protein of the invention and immunologically cross-reactive to said antibodies, for use in any of the immunological techniques described herein.

On page 31, line 2, please enter the following amendments:

Auxotroph Complementation Cloning of a ~~hemoglobin~~ Hemoglobin Receptor Gene from *Neisseria meningitidis*

On page 31, line 5, please enter the following amendments:

In order to identify *N. meningitidis* outer membrane receptor(s) involved in the uptake of ~~haemin~~ hemin and/or ~~haemoglobin~~ hemoglobin iron, an auxotroph complementation cloning strategy was used, similar to the approach previously taken to identify the *Y. enterocolitica* and *V. cholerae* hemin receptors (see Stojiljkovic and Hantke, 1992, *EMBO J.* 11: 43594367; Henderson and Payne, 1994, *J. Bacteriol.* 176: 3269-3277). This strategy is based on the fact that the outer membrane of Gram-negative bacteria is impermeable to hemin (McConville and Charles, 1979, J. Microbiol. 113:165-168) and therefore *E. coli* porphyrin biosynthesis mutants cannot grow on exogenously supplied hemin. If provided with the *N. meningitidis* outer membrane hemin receptor gene, the *E. coli* porphyrin mutant would be able to use exogenously supplied hemin as its porphyrin source.

On page 31, lines 19 & 21, please enter the following amendments:

A cosmid bank of *N. meningitidis* 8013 clone 6 DNA was prepared using conventional cosmid cloning methodologies (Sambrook *et al.*, 1989, *ibid.*). *N. meningitidis* bacterial DNA was partially digested by *Mbo*I, size fractionated on sucrose gradients and cloned into the *Bam*HI site of the cosmid vector pLAFR2 (Riboli *et al.*, 1991, *Microb. Pathogen.* 10: 393403). This cosmid bank was mobilized into the *E. coli* hemA aroB Rif^{r} recipient strain by triparental matings using a conjugal plasmid pRK2013::Tn9. The mating mixture was plated ~~onselective~~ on selective plates containing hemin chloride (50 mg/L), 0.1 mM 2,2'-dypyridil and rifampicin (100 mg/L). Several clones growing on exogenously supplied ~~haemin~~ hemin were isolated after an overnight incubation.

On page 33, line 2, please enter the following amendments:

The hemin utilization phenotype of these transformants was tested by re-introduction of the cosmids into naive *E. coli* hemA aroB cells and by monitoring the growth on hemin-supplemented plates. The ability of E. coli strains to utilize heme or hemoglobin as the sole iron source was tested as previously described (Stojiljkovic and Hantke, 1992, *ibid.*). Cells were grown on LB agar supplemented with 50μM deferoxamine mesylate (an iron chelating agent, obtained from Sigma ~~Chemicals~~ Chemical Co., St. Louis, Mo.). Filter discs (1/4 inches, Schleichner & Schuell, Inc., Keene, N.H.) impregnated with the test compounds (20μL of 5 mg/ml stock solutions unless otherwise stated) were placed on these plates. After overnight growth at 37° C. with 5 % CO_2, zones of growth around the discs were monitored. The iron-bound proteins tested in this assay (all obtained from Sigma Chemical Co.) were hemoglobin from human, baboon, bovine and mouse sources, bovine hemin, human lactoferrin (90% iron saturated), and human transferrin (90 % iron saturated, obtained from Boehringer Mannheim Biochemicals, Indianapolis, Ind.). A total of six hemin utilization positive cosmids were obtained using this protocol. Results using such assays are shown in Table II.

On page 33, line 13, please enter the following amendments:

Cosmid DNA from six hemin-utilization positive cosmids obtained as described in Example 2 were digested with *Cla*I, and the resulting fragments were cloned into *Cla*I-digested pSU(SK) vector (obtained from Stratagene, LaJolla, Calif.). One subclone, containing a 6 kb *Cla*I fragment from cosmid cos22 (the resultant plasmid was designated pIRS508), was determined to allow utilization of hemin and hemoglobin by *E. coli* hemA aroB assayed as described in Example 2. Another such clone, containing an 11 kb ~~*Cla*I fragment~~ *Cla*I fragment from cos44 was also determined to allow hemin utilization in these auxotrophic mutant cells. Restriction analysis and Southern hybridization indicated that the DNA fragments originating from cos22 and cos44 are unrelated.

On page 35, lines 11 & 19, please enter the following amendments:

The nucleotide sequence of the 3.3 kb *Bam*HI-*Hin*dIII DNA fragment carrying the hmbR gene and its promoter region was determined using the dideoxy chain termination method using a Sequenase 2.0 kit (obtained from U.S. Biochemicals, Cleveland, Ohio) and analyzed using a BioRad electrophoresis system, an AutoRead kit (obtained from Pharmacia, Uppsala, SE) and an ALF-370 automatic sequenator (Pharmacia, Uppsala, Sweden). Plasmid subclones for sequencing were produced by a nested deletion approach using Erase-a-Base kit (obtained from Promega Biotech, Madison, Wis.) using different restriction sites in the hmbR gene. The nucleotide and predicted

amino acid sequences of the hmbR gene are shown in ~~Figure 2~~ Figures 2A-2H

On page 36, lines 5 & 7, please enter the following amendments:

A typical Fur binding nucleotide sequence (designated ~~"fur box"~~ "Fur box") was found in the promoter region of the hmbR gene (~~Figure 2~~ Figures 2A-2H). Like hemin utilization in *Yersiniae* and *Vibrio*, hemin and hemoglobin utilization in *Neisseria* are known to be iron-inducible phenotypes (West and Sparling, 1985, *Infect. Immun.* 47: 388-394; Dyer *et al.*, 1987, *Infect. Immun.* 55: 2171-2175). In Gram-negative bacteria, conditional expression of many iron utilization genes is regulated by the Fur repressor, which recognizes a 19 bp imperfect dyad repeat (Fur-box) in the promoter regions of Fur-repressed genes. Recently, a genetic screen (FURTA) for the identification of Fur-regulated genes from different Gram-negative bacteria was described (Stojiljkovic *et al.*, 1994, *J. Mol. Biol.* 236: 531-545), and this assay was used to test whether hmbR expression was controlled in this way. Briefly, a plasmid carrying a Fur-box sequence is transformed into an *E. coli* strain (H1717) which possesses a Fur-regulated lac fusion in the chromosome. Expression of this Fur-regulated lac fusion is normally repressed. Introduction of a multicopy Fur-box sequence on the plasmid titrates the available Fur repressor thus allowing expression of the Fur-regulated lac fusion (this phenotype is termed FURTA positive). Using this screen, the smallest insert fragment from cosmid pIRS508 that produced a FURTA positive result was a 0.7 kb *Bam*HI-*Not*I DNA fragment carried on plasmid pIRS528 (see FIG. 1). This result indicated that the 0.7 kb *Bam*HI-*Not*I fragment carries a Fur-box and that gene expression from the hmbR promoter is controlled by a fur-type operon.

On page 36, line 11 & 18, please enter the following amendments:

Immediately downstream of the hmbR gene (at positions 2955 to 3000 bp in ~~Figure 2~~ Figures 2A-2H) was found a short nucleotide sequence that is 99 % identical to the flanking sequence of the PIII gene of N. gonorrhoeae (Gotschlich et al., 1987, J. Exp. Med. 165: 471482). The first 26 bp of this sequence represents one half of the inverted repeat (IR1) of the *N. gonorrhoeae* small repetitive element. This element is found in approximately 20 copies in both *N. gonorrhoeae* and *N. meningitidis* (Correia et al., 1988, *J. Biol. Chem.* 263: 12194-12198). The analysis of the nucleotide sequence from position 3027 to the *Cla*I (3984) restriction site (only the nucleotide sequence from *Bam*HI (1) to *Hin*dIII (3370) is shown in ~~Figure 2~~ Figures 2A-2H) indicated the presence of an IS1106 element (Knight et al., 1992, *Mol. Microbiol.* 6: 1565-1573). Interestingly, no nucleotide sequence similar to the IS1106 inverted repeat was found between the IRI element and the beginning of the homology to IS1106.

On page 37, line 2, please enter the following amendments:

A comparison of the transferrin (Thp1; Legrain *et al.*, 1993, *Gene* 130: 81-90), lactoferrin (LbpA; Pettersson *et al.*, 1993, *Infect. Immun.* 61: 4724-4733, and 1994, *J. Bacteriol.* 176: 1764-1766) and hemoglobin receptors (HmbR) from N. meningitidis is shown in ~~Figure 4~~ Figures 4A-4C. The comparison was done with the CLASTAL program from the PC/GENE program package (Intelligenetics, Palo Alto, Calif.). Only the amino-terminal and carboxyl terminal segments of the proteins are shown. An asterisk indicates identity and a point indicates similarity at the amino acid level. Lactoferrin and transferrin receptors were found to share 44.4% identity in amino acid sequence. In contrast, homology between these proteins and the hemoglobin receptor disclosed herein was found to be significantly weaker (22% amino acid sequence identity with lactoferrin and 21 % with transferrin receptor).

On page 37, line 15, please enter the following amendments:

It was known that the transport of iron-containing siderophores, some colicins and vitamin B12 across the outer membrane of E. coli depends on three cytoplasmic membrane proteins: TonB, ExbB and ExbD (~~Postle~~ Postle, 1990, *Mol. Microbiol.* 133: 891-898; Braun and Hantke, 1991, in Winkelmann, (ed.), Handbook of Microbial Iron Chelates, CRC Press, Boca Raton, Fla., pp. 107-138). In *Yersinia* and *Hemophilus*, hemin uptake was shown to be a TonB-dependent process (Stojiljkovic and Hantke, 1992, *ibid.*; Jarosik *et al.*, 1994, *Infect. Immun.* 62: 2470-2477). Through direct interaction between the outer membrane receptors and the TonB cytoplasmic machinery, the substrate bound to the receptor is internalized into the periplasm (Heller *et al.*, 1988, *Gene* 64: 147-153; Schoffler and Braun, 1989, *Molec. Gen. Genet.* 217: 378-383). This direct interaction has been associated with a particular amino acid sequence in membrane proteins associated with the TonB machinery.

On page 38, lines 1, 12 & 13, please enter the following amendments:

All TonB-dependent receptors in Gram-negative bacteria contain several regions of high homology in their primary structures (Lundrigan and Kadner, 1986, J. Biol. Chem. 261: 10797-10801). In the amino acid sequence comparison described in Example 5, putative TonB-boxes of all three proteins are underlined. The carboxyl terminal end of the HmbR receptor contains the highly conserved terminal phenylalanine and position 782 arginine residues thought to be part of an outer

membrane localization signal (Struyve et al., 1991, J. Mol. Biol. 218: 141-148; Koebnik, 1993, Trends Microbiol. 1: 201). At residue 6 of the mature HmbR protein, an amino acid sequence--ETTPVKA (SEQ ID NO.15)--is similar in sequence to the so called TonB-boxes of several Gram-negative receptors (Heller et al., 1988, ibid.). Interestingly, the putative TonB-box of HmbR has more homology to the TonB-box of the N. gonorrhoeae transferrin receptor (Cornelissen et al., 1992, J. Bacteriol. 174: 5788-5797) than to the TonB-boxes of E. coli siderophore receptors. When the sequence of the HmbR receptor was compared with other TonB-dependent receptors, the highest similarity was found with Y. enterocolitica HemR receptor although the similarity was not as high as to the Neisseria receptors.

In order to prove the TonB-dependent nature of the *N. meningitidis*, serotype C hemoglobin receptor, hmbR was introduced into exbB and tonB mutants of *E. coli* EB53, and the ability of the strains to utilize hemin and hemoglobin as porphyrin and iron sources was assessed. In these assays, both mutants of *E. coli* EB53 were unable to use hemin either as a porphyrin source or as an iron source in the presence of a functional hmbR (~~Table 2~~ Table II). The usage of hemoglobin as an iron source was also affected (~~Table 2~~ Table II). These results are consistent with the notion that the hmbR gene product, the *N. meningitidis* hemoglobin receptor protein of the invention, is TonB-dependent, since expression of this gene in TonB wild type *E. coli* supported the use of hemin and hemoglobin as sole iron source in the experiments disclosed in Example 2.

On page 39, line 16, please enter the following amendments:

As shown in the data presented in Table II, hmbR mediated both hemin and hemoglobin utilization when expressed in *E. coli*, but hemoglobin utilization was less vigorous than hemin utilization. To determine if the HmbR receptor has the same specificity in *N. meningitidis*, hmbR was inactivated with a 1.2 kb kanamycin cassette (aphA-3; Nassif *et al.*, 1991, *ibid.*) and transformed into wild-type *N. meningitidis* 8013 clone 6 (serotype C) cells. The inactivation of the chromosomal hmbR copy of the Km-resistant transformants was confirmed by Southern hybridization, as shown in FIG. 5. As can be seen from FIG. 5, wild-type *N. meningitidis* genomic DNA contains only one copy of the hmbR gene (lanes 1 and 3). In the Km^{γ} transformants, the size of the DNA fragments containing the wild-type gene has increased by 1.2 kb, which is the size of the Kan cassette (FIG. 5, lanes 2 and 4). When tested for its ability to utilize different iron-containing compounds, these mutant cells were found to be unable to use hemoglobin-bound iron, regardless of the source (human, bovine, baboon, mouse). The ability of the mutant to utilize hemoglobin-haptoglobin was not tested because the wild-type *N. meningitidis* strain is unable to use haptoglobin-haemoglobin

complex as an iron source. However, the mutant was still able to use hemin iron, lactoferrin- and transferrin-bound iron as well as citrate-iron (Table II). As the iron-containing component of hemoglobin is hemin, a hemoglobin receptor would be expected to be capable of transporting hemin into the periplasm. Indeed, the cloning strategy disclosed herein depended on the ability of the cloned meningococcal receptor to transport hemin into the periplasm of *E. coli*. These results strongly suggest that *N. meningitidis* has at least two functional receptors that are involved in the internalization of hemin-containing compounds. One is the hemoglobin receptor described herein, which allows the utilization of both hemin and hemoglobin as iron sources. The other putative receptor in *N. meningitidis* is a hemin receptor which allows utilization of only hemin. This schema is also consistent with the isolation of several cosmid clones that allow *E. coli* EB53 to utilize hemin. DNAs from these cosmids do not hybridize with ~~our~~ the hmbR ~~probe;~~ probe, indicating that these clones encode a structurally-distinct receptor protein capable of transporting hemin into the periplasm of *N. meningitidis* cells.

On page 41, lines 3, 5, 7, and 10, please enter the following amendments:

5'-AAACAGGTCTCGGCATAG-3' (sense primer) (SEQ ID No.:~~15~~ 11)or

5'-CGCGAATTCAAACAGGTCTCGGCATAG-3' (sense primer) (SEQ ID No.:~~16~~ 12) and

5'-CGCGAATTCAAAAACTTCCATTCCAGCGATACG-3' (antisense primer) (SEQ ID No.:~~17~~ 13) or

5'-TAAAACTTCCATTCCAGCGATACG-3' (antisense primer) (SEQ ID No.:~~18~~ 14)

On page 42, line 11, please enter the following amendments:

As a result of these experiments, three clones encoding the hemoglobin receptor genes from N. meningitidis serotypes A and B and *N. gonorrhoeae* MS11A were cloned and the sequence of these genes determined. The nucleic acid sequence for each of these genes are shown in ~~Figure 7~~ Figures 7A-7I (*N. meningitidis*, ~~serotype A~~ serotype B), ~~Figure 8~~ Figures 8A-8I (*N. meningitidis*, ~~serotype B~~ serotype A) and ~~Figure 9~~ Figures 9A-9I (*N. gonorrhoeae* MS11A).

On page 42, lines 16 & 24, please enter the following amendments:

The degree of homology between the cloned hemoglobin receptors from the different *N. meningitidis* serotypes and *N. gonorrhoeae* MS11A was assessed by nucleic acid and amino acid sequence comparison, as described in Example 5 above. The results of these comparisons are shown in ~~Figures 10~~ Figure 10 and ~~Figure 11~~ Figures 11A-11D, respectively. Hemoglobin receptor genes from the three *N. meningitidis* serotypes and *N. gonorrhoeae* MS11A were found to be from 86.5% to 93.4% homologous; the most homologous nucleic acids were *N. meningitidis* serotypes B and C, and the most divergent nucleic acids were *N. meningitidis* serotype B and N. gonorrhoeae MS11A (Figure 10 and Table III). Hemoglobin receptor proteins from all four *Neisseria* species showed a high degree of homology to the other members of the group, ranging from 87% homology between the hemoglobin receptor proteins from *N. gonorrhoeae* MS11A and *N. meningitidis* serotype B to 93% homology between hemoglobin receptor proteins from *N. meningitidis* serotypes A and B (~~Figure 11~~ Figures 11A-11D). In this comparison, all four receptors were found to share 84.7% amino acid sequence identity, and up to 11.6% sequence similarity (*i.e.*, chemically-related amino acid residues at homologous sites within the amino acid sequence). The non-conserved amino acids were found clustered in the regions of the amino acid sequence corresponding to the external loops in the predicted topographical structure of the hemoglobin receptor proteins.